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The Prostate 28:65–69 (1996)

Presentation of Prostate Tumor Antigens by Dendritic Cells Stimulates T-Cell Proliferation and Cytotoxicity

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ABSTRACT: Dendritic cells (DCs) are "professional" antigen-presenting cells capable of stimulating T-cell proliferation and cytotoxicity when loaded with and presenting specific antigens, including tumor antigens. We demonstrated the stimulation of an autologous cytotoxic T-cell response elicited by DC loaded with autologous tumor cell lysate derived from primary prostate tumor. A candidate tumor antigen is prostate-specific membrane antigen (PSMA), which is overexpressed in prostate cancer patients. We identified a HLA-A2 motif in PSMA, isolated patient DC, loaded peptide into DC, and stimulated autologous T cells to proliferate. The ability to use DC for presentation of either tumor or peptide antigen in an HLA-restricted fashion in order to stimulate T-cell proliferation and cytotoxicity demonstrates the potential of this technology for development of a prostate cancer vaccine.

KEY WORDS: **dendritic cells, T-cell response, prostate-specific membrane antigen**

INTRODUCTION

Cancer immunotherapy studies involve various strategies to enhance immune responses against neoplastic cells [1]. An effective "cancer vaccine" that activates tumor-specific T cells in vivo requires key components, including efficient antigen-presenting cells (APCs) and tumor-specific antigen(s). Dendritic cells (DCs) are suggested as the most efficient APCs to activate both naive and memory T cells [2]. Analyses of the potential of DCs for use as APCs in cancer immunotherapy were previously limited due to the low numbers of DCs that can be isolated, particularly from cancer patients whose immune system may have been compromised due to previous therapy [2–4]. Our laboratory recently demonstrated that DCs can be propagated in vitro from peripheral blood mononuclear cells (PBMCs) of prostate cancer patients, many of whom are in clinical stages D_1 or D_2 and have undergone radiation therapy [5]. The number of DCs recovered after a 7-day culture was 20- to 50-fold higher than those isolated directly from peripheral blood, and these numbers are comparable to findings of previous studies with healthy individuals [5]. Cultured patients' DCs were capable of presenting soluble antigens, including tetanus toxoid and the lysate of a prostate cancer cell line (LNCaP), to autologous T cells in vitro [5].

In order to evaluate further the potential of DCs in prostate cancer immunotherapy, we examined the ability of patients' DCs to present additional prostate cancer antigens. Autologous tumor cells, cultured from a prostatectomy specimen, were used as one of the sources of antigen in the present study. Lysate of these tumor cells were introduced to autologous DCs and presented to autologous T cells in vitro. The ca-

Received for publication August 1, 1995; accepted September 1, 1995.

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pacity of the T-cell population generated in this manner to recognize and lyse specific targets, including the autologous prostate tumor cells, is evaluated.

T cells recognize processed peptide antigens bound to products of the major histocompatibility complex (MHC) [6]. Previous studies demonstrated that peptides bound to class I MHC molecules exhibit a typical length of 9±1 amino acids and carry "anchor" residues crucial for binding, typically in position 2 and the C-terminal position [7–9]. The identification of these motifs allows for rapid prediction of MHC class I-binding peptides from proteins of known amino acid sequences.

HLA-A2.1 is expressed by a large proportion of the population [10]. In the current study, we selected a 9-amino acid peptide, which possesses the HLA-A2.1-binding motif, from the amino acid sequence of prostate-specific membrane antigen (PSMA) as a prostate antigen to be presented by autologous DCs. PSMA is a specific prostate antigen recognized by monoclonal antibody 7E11.C5 [11]. This antibody is being used for in vivo imaging in ongoing clinical trials to detect metastatic prostatic carcinoma [12]. Levels of PSMA are elevated in the serum of hormone refractory advanced prostate cancer patients. PSMA is also found in normal male serum and seminal fluid and in prostate epithelial cells [13–15]. This report describes the capacity of autologous DCs from prostate cancer patients to present the PSMA peptide to activate patients' T cells in vitro.

MATERIALS AND METHODS

Cell Lines and Reagents

AL-132, a prostate tumor cell line, was a generous gift from Dr. Richard Ostenson and Steven Loop from the Veteran's Administration Hospital, Tacoma, Washington. Other prostate tumor cell lines were developed in our laboratory from radical prostatectomy specimens. Granulocyte–macrophage colony-stimulating factor (GM-CSF), recombinant human interleukin-2 (IL-2), and interleukin-4 (IL-4) were generous gifts from Amgen (Thousand Oaks, CA). PSMA 4-12 peptide (LLHETDSAV) was synthesized by Genemed (San Francisco, CA). This peptide was reconstituted in water to reach a concentration of 10 mg/ml, sterile filtered, and stored in a −80°C freezer in small aliquots.

DC Culture from Peripheral Blood Mon nuclear Cells

DC isolation and culture were obtained from patients pre- and post-operatively, and performed as described previously [5]. In short, peripheral blood was drawn and subjected to Lymphoprep (GIBCO–BRL, Gaithersburg, MD) density-gradient centrifugation. The PBMC isolated were plated in 24-well plates (10^6–10^7 cells/well) and were incubated in a humidified incubator (37°C, 5% CO_2) for 90 min. Nonadherent cells were removed with the supernatant, and the wells were washed gently with warm (37°C) OPTI-MEM medium (GIBCO-BRL, Gaithersburg, MD), and 5% fetal calf serum (FCS). Dendritic cell propagation medium (DCPM: OPTIMEM supplemented with 5% FCS, 500 U/ml GM-CSF and 500 U/ml IL-4) was added to the adherent cells (1 ml/well). These cells were cultured for 4–6 days before being subcultured 1:3 in DCPM.

Generation of T-Cell Responses Against Autologous Tumor Cell Lysate In Vitro

Prostate tumor cells including AL132, were cultured from fresh radical prostatectomy specimens. Tumor cells were harvested and washed in sterile phosphate-buffered saline (PBS). Tumor cell lysate was prepared by repeated freezing and thawing in liquid nitrogen and a 37°C water bath, respectively. The lysate was introduced to cultured autologous DCs from the prostate cancer patient by the method of osmotic lysis of pinosomes as described previously [16]. One hundred thousand autologous tumor lysate-treated DCs were incubated with 5×10^6 autologous PBMC in T-cell medium (TCM) consisting of RPMI 1640, Hepes, 2-mercaptoethanol, L-glutamine, and penicillin-streptomycin, supplemented with 10% heat-inactivated human AB serum (Sigma, St. Louis, MO). On day 5, proliferating T cells were subcultured 1:4 in the presence of TCM + IL-2. The culture was maintained by weekly subculture. Mitomycin C-treated autologous DCs treated with autologous tumor cell lysate as described above were added to the culture every other week.

^{51}Cr Release Assay

Target cells were labeled with ^{51}Cr, and 10^4 cells were incubated with effector cells in a 96-well plate at various effector to target cell ratios for 4 hr at 37°C. In some experiments, after incubation, supernatants were removed and monitored for ^{51}Cr, specific release of ^{51}Cr was calculated by

$$\text{Specific } {}^{51}\text{Cr released} = \frac{(\text{experimental counts} - \text{spontaneous counts})}{(\text{maximal counts} - \text{spontaneous counts})} \times 100\%.$$

In Vitro Generati n f Autolog us T-Cell Response Against PSMA 4-12 Peptide and T-Cell Pr liferati n Assays

One million prostate cancer patients' PBMC were plated in microtiter plates in TCM and 1 U/ml recombinant human IL-2. DCs were harvested, resuspended in TCM and 20 μg/ml PSMA 4–12, and incubated for 4–6 hr. Peptide-pulsed DCs were subsequently treated with 50 μg/ml mitomycin C (Sigma, St. Louis, MO), washed, and counted. Ten thousand mitomycin-C-inactivated peptide-pulsed autologous DC were added to the wells and cultured in a 37°C, 5% CO_2 humidified incubator for 5–7 days. One μCi ^{3}HT-dR was added to every well. The proliferation assay was harvested 24 hr, later and the ^{3}H-TdR incorporated was analyzed, using a liquid scintillation counter.

RESULTS

In Vitro Generation of T-Cell Response Against Autologous Prostate Tumor Cell Lysate Presented by Autologous DCs

Prostate tumor cell lysate was inserted into the cytoplasm of cultured autologous DCs, using the technique of osmotic lysis of pinocytic vesicles. Autologous PBMCs were subsequently added and cultured as described in the Materials and Methods. After 2 cycles of restimulation, the in vitro-generated T cells preferentially lysed autologous DCs after introduction of tumor cell lysate in a ^{51}Cr release assay (Fig. 1). Furthermore, these T cells were capable of lysing autologous prostate tumor cells, presumably through TCR recognition of endogenous tumor-specific epitopes (Fig. 2). An increasing degree of cytotoxicity was observed after these T-cell populations underwent another cycle of restimulation, suggesting the expansion of cancer-specific cytotoxic cells (Fig. 2).

Specific Proliferation of Autologous T Cells In Vitro in the Presence of Autologous DCs Pulsed With A Prostate-Specific Peptide

The peptide LLHETDSAV corresponds to positions 4–12 of the predicted PSMA amino acid sequence. This peptide was selected for this study based on the binding motif of peptides (9–10-amino acid long) that bind HLA-A2.1, i.e., the presence of "anchor" residues: leucine (L) at position 2 and valine (V) at position 9. Upon presentation of peptide-pulsed autologous DCs to PBMC from prostate cancer patients, increases in ^{3}H-TdR incorporation compared to PBMC cultured in the presence of autologous DCs alone were observed, suggesting specific response to the PSMA peptide (Fig. 3). Significant increase in cell proliferations were observed only in

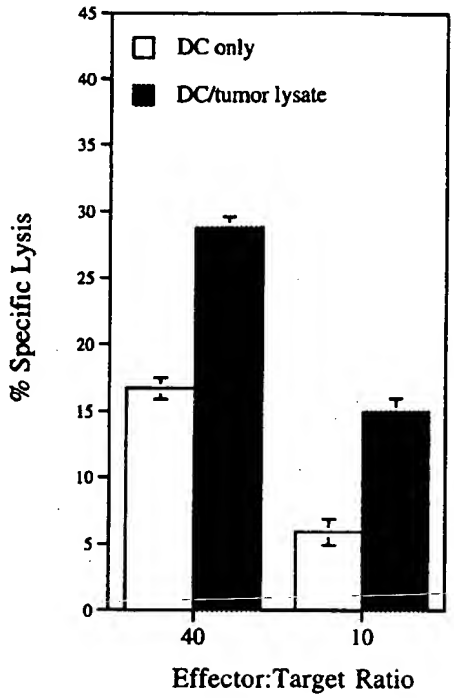

Fig. 1. In vitro-generated T cells against autologous tumor cell lysate preferentially lyse autologous DCs pulsed with tumor cell lysate. A 4-hr ^{51}Cr release assay was performed with in vitro-generated T cells against autologous tumor lysate (24 days in culture) as effector cells, and autologous DC (DC only) or autologous DC pulsed with tumor cell lysate (DC/tumor lysate) as target cells as described in Materials and Methods. The values are the mean ±SEM from three separate cultures.

cultures generated from HLA-A2$^+$ patients, demonstrating that the in vitro generated T-cell responses were HLA-A2$^+$ restricted (Fig. 3).

DISCUSSION

The current study demonstrates the capacity of in vitro-propagated autologous DCs from prostate cancer patients to present prostate antigens to autologous PBMC resulting in proliferation of specific T cells. One source of prostate antigens examined in this study is a crude lysate from autologous tumor cells. Autologous T cells proliferated upon culture with tumor cell lysate-loaded autologous DCs. After several cycles of restimulation, proliferating T cells raised against autologous prostate tumor cell lysate/autologous DCs exhibited cytotoxicity for autologous DCs treated with the tumor lysate.

A majority of studies suggest that T helper cells (T_H, CD4$^+$) predominate the in vitro T-cell responses to exogenous protein antigen presented by DCs [17,18]. In the present study, total cell lysate, prepared from prostate tumor cells cultured from a prostatectomy specimen, was introduced into the DC cytosol using the technique of osmotic lysis of pinosomes as previ-

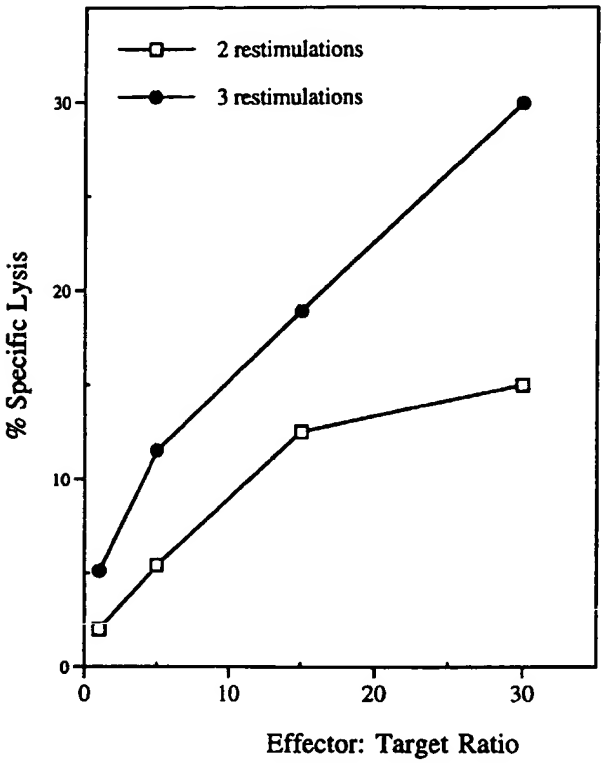

Fig. 2. In vitro-generated T cells against autologous tumor cell lysate lyse autologous tumors. T cells generated against autologous tumor cell lysate-pulsed DCs—17 days, 2 restimulations (□) or 29 days, 3 restimulations (●)—were subjected to a ^{51}Cr release assay as described in Materials and Methods. Target cells were autologous prostate tumor cells, from which culture the lysate was prepared.

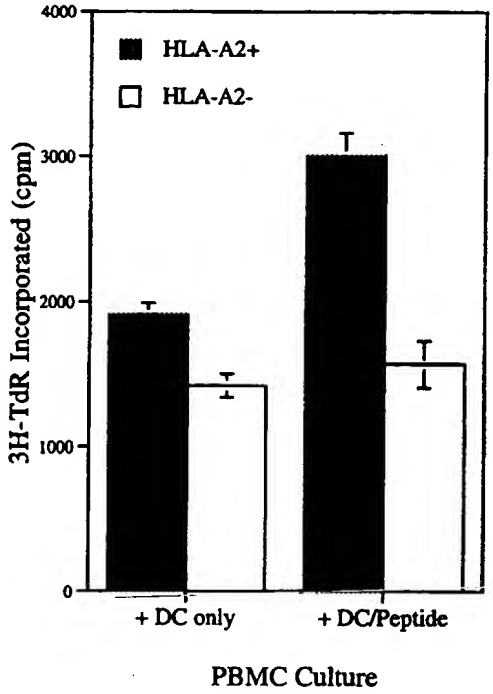

Fig. 3. In vitro generation of autologous T-cell response against a PSMA peptide using autologous DCs as APCs. Autologous PBMC from an HLA-A2$^+$ and HLA-A2$^-$ prostate cancer patients were cultured in the presence of mitomycin-C-treated autologous DCs pulsed with the PSMA 4–12 peptide (+DC/peptide), mitomycin-C-treated autologous DCs only (+DC only), or medium only, and were subjected to a ^3H-TdR incorporation assay as described in Materials and Methods. The number of counts per minute (cpm) from cultures of PBMC in the presence of medium only was averaged and subtracted from the cpm from (+DC/peptide) and (+DC only). The subtracted values presented are the mean ±SEM from three separate cultures.

ously reported [16,19]. Our cytotoxicity results suggest that this method of antigen-loading of DCs was effective in eliciting CTLs in vitro, consistent with a previous report by Mehta-Damani et al. [19]. Furthermore, the T-cell populations elicited in the presence of autologous prostate tumor cell lysate-loaded DCs lyse autologous prostate tumor cells in vitro, presumably as a result of T-cell recognition of endogenously processed tumor antigens. This result indicates that T cell elicited in this manner could be useful for specific T-cell immunotherapy for prostate cancer.

CTL epitopes are endogenously processed peptides bound to MHC class I molecules. The elucidation of MHC class I consensus binding motifs has made it possible to predict potential tumor-specific CTL peptide epitopes from the amino acid sequence of tumor antigens [7–9]. For example, peptides that bind HLA-A2.1 with high affinity are 9–10 amino acids long and possess "anchor" residues leucine and valine at positions 2 and 9, respectively. In this study, we selected a peptide, with HLA-A2.1-motif from the prostate antigen PSMA. T cells were elicited in vitro when autologous PBMC were cultured in the presence of DCs from an HLA-A2$^+$ prostate cancer patient pulsed with the PSMA 4–12 peptide (LLHETDSAV). By contrast, no significant increase of proliferation was observed in experiments with cells from an HLA-A2$^-$ patients, compared to control experiments in the presence of DCs alone, suggesting that the in vitro activation of T cells was HLA-A2-restricted. Approximately 40% of patients with prostate cancer exhibit HLA-A2 phenotype and, thus, can potentially display the PSMA 4-12 peptide bound to HLA-A2 molecules on the surface of their prostate tumor cells [20]. Further restimulations of the in vitro generated T cell populations are currently being conducted to characterize their cytotoxic properties against HLA-A2$^+$ prostate tumor cells. Additional peptides that could potentially bind other MHC class I molecules also exist within the PSMA amino acid sequence, making it possible to pursue a more complete study that covers most patients with prostate cancer.

DCs have been shown to play a critical role in the

initiation of T-cell responses [2,21]. Introduction of DCs pulsed with cancer antigens may be an effective way of generating a tumor-specific T-cell response in vivo and thus has a potential as a cancer vaccine. The low number of DCs that can be isolated directly from any single tissue has been solved by several investigators who have discovered methods to propagate DCs in vitro [5,17,22–25]. Furthermore, we have previously reported that large numbers of functionally active DCs can be cultured from immune compromised cancer patients [5]. We demonstrated that using patients' DCs as APCs, autologous T cells could be elicited in vitro against prostate antigens [5]. Our next step, which is underway, is a phase I clinical trial administering cultured DCs pulsed with prostate antigens back to advanced prostate cancer patients.

ACKNOWLEDGMENTS

The authors thank Mr. Robert Barren III, Ms. Sheila Erickson, and Ms. Victoria Bowes of the Pacific Northwest Cancer Foundation for their skillful technical assistance. This work was supported in part by grants from CaP Cure and the Phi Beta Psi Sorority Cancer Research Fund.

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